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Functionality of Extrusion—Texturized Whey Proteins[1]

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ABSTRACT

Whey, a byproduct of the cheesemaking process, is concentrated by processors to make whey protein concentrates (WPC) and isolates (WPI). Only 50% of whey proteins are used in foods. In order to increase their usage, texturizing WPC, WPI, and whey albumin is proposed to create ingredients with new functionality. Extrusion processing texturizes globular proteins by shearing and stretching them into aligned or entangled fibrous bundles. In this study, WPC, WPI, and whey albumin were extruded in a twin screw extruder at approximately 38% moisture content (15.2 ml/min, feed rate 25 g/min) and, at different extrusion cook temperatures, at the same temperature for the last four zones before the die (35, 50, 75, and 100°C, respectively). Protein solubility, gelation, foaming, and digestibility were determined in extrudates. Degree of extrusion-induced insolubility (denaturation) or texturization, determined by lack of solubility at pH 7 for WPI, increased from 30 to 60, 85, and 95% for the four temperature conditions 35, 50, 75, and 100°C, respectively. Gel strength of extruded isolates increased initially 115% (35°C) and 145% (50°C), but gel strength was lost at 75 and 100°C. Denaturation at these melt temperatures had minimal effect on foaming and digestibility. Varying extrusion cook temperature allowed a new controlled rate of denaturation, indicating that a texturized ingredient with a predetermined functionality based on degree of denaturation can be created.
(**Key words:** extrusion, texturization, whey proteins, functionality)

Abbreviation key: WLAC = whey lactalbumin, **WPI** = whey protein isolate.

INTRODUCTION

Though new products incorporating whey proteins, such as sports drinks, are being developed, innovation in process and product development is still needed (American Dairy Products Institute, 2000). Fortifying snacks with whey proteins could provide a particularly attractive outlet for surplus whey proteins. This practice has been limited, however, due to known adverse textural effects when the whey protein concentrate supplementation is greater than 10% of the main starch component (Kim and Maga, 1987). It has been the hope that successful incorporation of whey into extruded products will increase use of whey products and improve the nutrient density of snacks by increasing the protein content. However, using nontexturized whey proteins has not been successful in improving functional qualities of coextruded puffed products, especially their expansion and crunchiness (Singh et al., 1991).

Adding proteins to extruded starch-based snacks increases the number of sites for crosslinking, but shorts the starch matrix, resulting in tough, nonexpanded crusts (Martinez-Serna and Villota, 1992). The source, concentration, and processing conditions of the starting whey proteins are significant factors, since partial or complete denaturation affects their structure and leads to changes in crosslinking and complexing of the whey proteins during extrusion (Aboagye and Stanley, 1987). Proteins can be pretexturized or denatured before adding to the extruder to create a better matrix, which could lead to improved functionality (Kester and Richardson, 1983; Mohammed et al., 2000).

Extruders, with their shearing screws operating at varying speeds, impart significant structural changes to food components including proteins (Harper, 1981). A demonstrated benefit of high shear processes is the ability to change the molecular structure of proteins (Kollengode et al., 1996; Batterman-Azcona and Hamaker, 1998). Though protein denaturation by thermal extrusion is not well established (Taylor and Fryer, 1994), whey proteins can be modified using chemicals, heat, or by shear in the extruder (Kim and Maga, 1987). Chemical treatment alone alters the reactive groups of the amino acids, resulting in changes in the noncovalent forces that influence conformation such as van der Waals forces, electrostatic interactions, hydrophobic interactions, and hydrogen bonding (Kester and Richardson, 1984). Heating and shear alter the conformational

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structure of the protein through partial denaturation of the protein, thereby exposing groups that are normally concealed in the native protein (Kim and Maga, 1987).

To increase utilization of whey proteins, effort is needed to improve their functionality in the presence of other components such as starches, flours, and other nondairy proteins. This can be accomplished by first texturizing the whey proteins through denaturing their globular structure, or unfolding and realigning the proteins, before inclusion with other matrices. Therefore, our objectives in this work were to texturize and modify the functionality of whey proteins to create new uses for them.

MATERIALS AND METHODS

Whey protein concentrate (ALACEN 834) and lactalbumin (ALATAL 825) were purchased from New Zealand Milk Products, Inc. (Santa Rosa, CA). Whey protein isolate (**WPI**; PROVON 190) was purchased from Glanbia Ingredients. The compositions were as follows: whey protein concentrate: moisture 2.8%, protein 83.6%, fat 0.8, ash 3.3%, and carbohydrate by difference; whey lactalbumin (**WLAC**): moisture 5.5%, protein 89.9%, fat 3.8, ash 0.5%, and carbohydrate by difference; WPI: moisture 2.8%, protein 89.6%, fat 25, ash 3.3%, and carbohydrate by difference.

A ZSK-30 twin screw extruder (Krupp Werner Pfleiderer Co., Ramsey, NJ) with a smooth barrel was used. The extruder has nine zones, and the effective cooking zones 6, 7, 8, and 9 were set to the same temperature for each test. To achieve different melt temperatures the cooking zones were set to the same barrel temperature 35, 50, 75, or 100°C, respectively. Zones 1 to 3 were set to 35°C and zones 4 and 5 were set to 75°C. For example, the profile for extrusion cook temperature 35 was: 35, 35, 35, 75, 75, 35, 35, 35, 35. Melt temperature was monitored behind the die. The die plate was fitted with two circular inserts of 3.18 mm diameter each. The screw elements were selected to provide low shear at 300 rpm; the screw profile was published by Onwulata et al. (1998). Feed was conveyed into the extruder with a series 6300 digital feeder, type T-35 twin screw volumetric feeder (K-tron Corp., Pitman, NJ). The feed screw speed was set at 600 rpm, corresponding to a rate of 3.50 kg/h. Water was added into the extruder at the rate of 1.0 L/h with an electromagnetic dosing pump (Milton Roy, Acton, MA). Samples were collected after 25 min of processing, freeze-dried overnight in a VirTis Freeze Mobile 12XL Research Scale Freeze Dryer (Gardiner, New York), and stored at 4.4°C until analyzed. The experiments were performed in triplicate.

Analysis of variance was used to identify differences in physical properties at various processing conditions. Duncan's multiple range test was used for mean separation, and correlation coefficients were calculated. The Statistical Analysis System (SAS) package was used (SAS Institute Inc., Cary, NC) in all cases. Significance was defined as $P = 0.05$.

Moisture was determined by the AOAC Official Method 925.10. Extrudate samples weighing approximately 1.5 g were dried in a vacuum oven at 100°C overnight (AOAC, 2000).

Ash was determined by the AOAC Official Method 923.03. Ash was determined for each sample using 3 g assayed in a Muffler furnace at 550°C for 16 h; percentage of ash was calculated.

Fat was determined using the AOAC Official Method 30-25. One gram of extrudate was placed in an Erlenmeyer flask and 1 ml of sulfuric acid and 4 ml water was added to the flask. The samples were mixed gently and after 60 min were transferred to a 60-ml separatory funnel using 25 ml of dichloromethane:methanol solution (1:1). Extrudate sample were shaken and allowed to separate for 15 min. The bottom layer was drained into a weighing pan and then evaporated, and the amount of fat was determined (AACC, 1995).

Protein was determined with 0.2 g of extrudate analyzed with the LECO Protein Analyzer Model FP2000 (LECO Corporation, St. Joseph, MI). Percent protein was calculated with the N conversion factor 6.38 for whey protein.

Gel strength was measured by Bloom determinations with a TA-XT2 Texture Analyzer as described by Ju and Kilara (1998). A 12% WPI solution was made (3.204 g of ground freeze-dried sample mixed with 26.7 ml of deionized water and 3.3 ml of 0.03 M $CaCl_2$), and allowed to sit for 15 min in a 50- × 70-mm cylindrical jar. The sample was heated to 80°C for 30 min in a water bath, cooled in an ice bath for 15 min, and then stored overnight at 4°C. The specimen was thawed at 25°C in a 50% relative humidity room. Gel strength was determined with a TA-XT2 Texture Analyzer running a penetration test with a 30-mm analytical probe to a depth of 6 mm at the rate of 1 mm/s. The weak gels were easily deformed with evidence of syneresis.

Protein insolubility was determined with 1.0 g of ground freeze-dried extrudate sample mixed with 90 ml of deionized water. The protein suspension was stirred at 125 rpm, at pH 7.0 for 2 h. The suspension was centrifuged for 20 min, and the supernatant was freeze-dried overnight. The LECO Protein Analyzer Model FP2000. was used to analyze the solids from the supernatant for protein content. Protein insolubility (denaturation) was calculated as described by Kilara

(1984) as: [% total protein – % soluble protein = % insoluble (denatured)].

Foam volume and stability of extruded proteins were determined by heating 2.3-g samples mixed with 35 ml of deionized water to 60°C for 15 min. The slurry was then whipped for 15 s in Waring Lab Micronizer FPC70 (Waring Products Division, New Hartford, CT), then transferred to a 100-ml graduated cylinder where the foam volume was read initially, and then every 5 min for 1 h. Foam stability (foam capacity at specific time) over the 1-h period was calculated.

Protein digestibility was determined with 10 ml of extrudate sample dissolved in distilled water. The pH was then adjusted to 8.0 with 0.1 *N* NaOH or HCl. One milliliter of freshly prepared enzyme stock solution (1.6 mg/ml trypsin, 3.1 mg/ml Chymotrypsin, and 1.3 mg/ml aminopeptidase) was added to the protein suspension at 37°C. The pH after 10 min was recorded with a portable pH meter (IQ Scientific Instruments, Inc., San Diego, CA), and a Tris/HCl buffer containing 2.0% SDS (wt/vol) and 0.1% mercaptoethanol (vol/vol) was added to the protein solution which was immediately heated to 90°C to determine the enzymatic reaction. Samples were then analyzed by quantitative gel electrophoresis. The percent protein digestibility was calculated by the following equation: %digestibility = 210.46 B 18.10(X), where X is the pH (Ju and Kilara, 1998).

For SDS-PAGE assay, samples were vortexed and dissolved in 20 m*M* Tris/HCl, 5 m*M* EDTA, 2.5% SDS with and without 5.0% 2-mercaptoethanol at pH = 8.0 then heated in boiling water for 2 min. Bromophenol blue was added to about 0.1% concentration. The samples were at 2 mg/ml concentration. Phast gels (Amersham Pharmacia Biotech, Uppsala, Sweden) were run according to the procedures given by the manufacturer for SDS 20% homogeneous gels. The 6-lane (4 μl per lane) sample applicators were used. Protein staining used the Coomassie blue procedure given by the manufacturer (Farrell et al., 1998).

For fine structure, transmission electron microscopy was done of thin sections made from epoxy-embedded samples. Millimeter-sized pieces of coarsely ground, freeze-dried segments of ribbons of the extrudates were immersed in 2.5% glutaraldehyde in 0.1 *M* imidazole buffer solution (pH 6.8) and stored in sealed vials at 4°C. For embedding and thin sectioning, the segments were washed in imidazole buffer, immersed in 2% osmium tetroxide in 0.1 *M* imidazole buffer for 2 h at room temperature, washed in distilled water, and gradually dehydrated in a series of ethanol solutions and propylene oxide for 1 h. Samples were then infiltrated with a 1:1 mixture of propylene oxide and epoxy resin mixture overnight and finally embedded in epoxy resin. Thin sections were cut and stained with 2% uranyl acetate, and lead citrate solutions. The transmission electron microscopy was done in the bright field mode using a model CM12 electron microscope (FEI/Philips, Hillsboro, OR). Average spacings of electron density, corresponding to fine structure in the extrudates, were estimated from the intensity distribution in Fourier transforms computed from digital images made from transmission electron microscopy photographic negatives recorded at 45,000×. Negatives were digitized using a SprintScan 45 film scanner (Polaroid Corp., Cambridge, MA) and square areas of 2.8 MB images (512 × 512 pixels) were transformed after flattening, adjustment of brightness and contrast and one cycle of a low pass filter using a 3 × 3 pixel kernel in Image Pro Plus software (Media Cybernetics, Silver Spring, MD). Line profiles of the radial distribution of intensity in the Fourier transforms were made, and reciprocal spacings were calculated based on the location of orders of peaks in transforms of a line grating with an equivalent spacing of 22 nm.

Table 1. Extrusion melt temperatures of whey proteins.

Product	Melt temperature (EC)	Preextrusion (%)	Postextrusion (%)
WPC80[1]	70[b]	40.9[b]	59.9[b]
WLAC[2]	75[a]	68.7[a]	94.4[a]
WPI[3]	74[a]	28.0[a]	94.8[a]

[a,b,c]Means with different letters within a column are significantly ($P < 0.05$) different.

[1]WPC80 = Whey protein concentrate, 80% protein.

[2]WLAC = Whey lactalbumin.

[3]WPI = Whey protein isolate: number reported is mean of three samples. The preextrusion and postextrusion numbers are percent insoluble protein.

For scanning electron microscopy, a layer of dry powder particles was adsorbed onto conductive carbon adhesive tabs glued to aluminum specimen stubs (Electron Microscopy Sciences, Ft. Washington, PA), and the surface was coated with a thin layer of gold in a model Scancoat Six sputter coater (BOC Edwards, Wilmington, MA). Images of the powder particles were made with a model JSM 840A scanning electron microscope (JEOL USA, Peabody, MA) operating in the secondary electron imaging mode and integrated with a digital image workstation, model Imix1 (Princeton Gamma-Tech, Princeton, NJ).

RESULTS AND DISCUSSION

Extruding whey proteins at the cook temperature of 75°C resulted in varying degrees of melt temperatures and denaturation for the different products (Table 1). Following extrusion, whey protein concentrate was the least denatured and WLAC and WPI were significantly

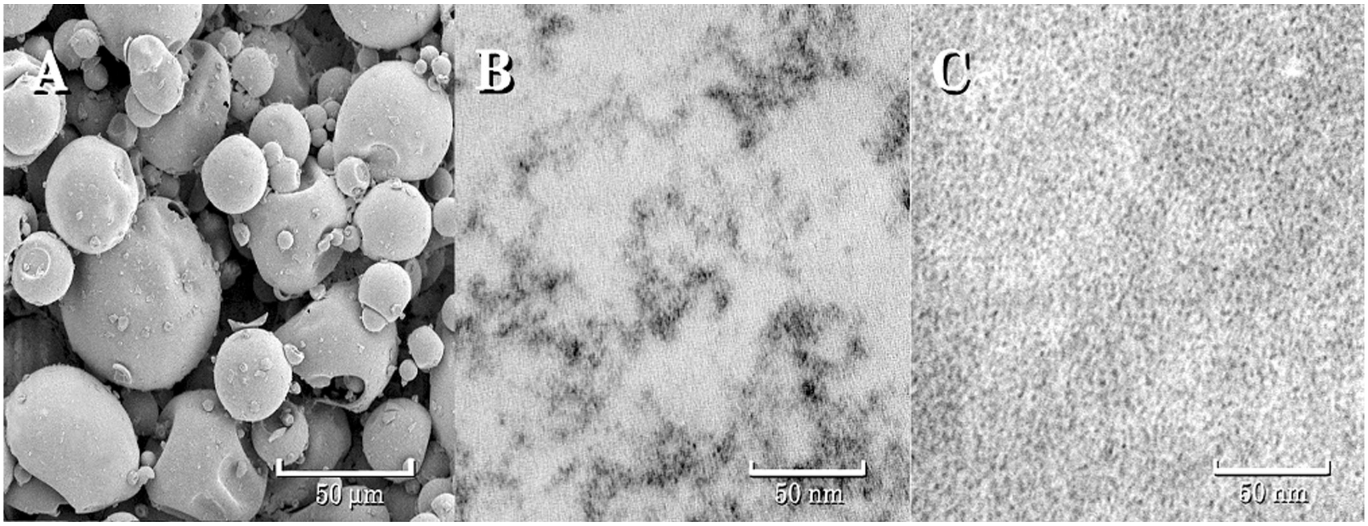

Figure 1. Electron micrograms of whey protein isolates (WPI). (A) Scanning microscopy was used to examine dry powder. (B) The nonextruded WPI paste (40% moisture) was embedded, stained with uranyl acetate and sections examined by transmission electron microscopy; (C) Extruded (100°C) WPI (40% moisture) treated as in (B).

($P < 0.05$) more denatured. Although WPI and WLAC were equally denatured, because of the wider spread in insolubility between the initial and final WPI values, from 28 to 94.8%, further experiments were conducted with WPI.

The effect of extrusion cooking on denatured proteins was examined by electron microscopy. Changes in the microstructure of WPI and the ultrastructure of the denatured proteins are presented in Figure 1. The microstructure of the dry powders, examined by scanning electron microscopy, reveal particles ranging from 10 to 50 μm in diameter (A). Transmission electron microscopy (B) shows the release of protein at the edge of powder particles after brief exposure to water typical of initial mixing in the extruder. Here irregular strings and granules, corresponding to molecular aggregates, ranging from less than 10 nm to over 200 nm can be seen (B). In contrast, the ultrastructure of extruder-denatured insoluble whey protein shows a closely packed arrangement of electron dense particles, typical of denatured protein matrix, ranging from approximately 2 to 6 nm in diameter (C).

As the whey proteins denature, they become insoluble and aggregate (Walstra et al., 1999). Changes in globular protein structure occur at or above 50 and 80°C for α-lactalbumin and β-lactoglobulin, respectively (Farrell et al., 2002; Hong and Creamer, 2002). However, with the addition of shear in the extruder, significant unfolding (denaturation) occurred at 50°C. Other conditions that favor denaturation are pH conditions lower 4.6 for whey proteins (Harwalker, 1979). WPI extruded at cook temperature 50°C or above, denatured significantly ($P < 0.05$) with increased cook temperature, increasing in order of magnitude every 25°C. The pH of the suspended protein remained stable as extrusion temperature increased, but measurable N (protein) increased as shown in Table 2. The heat denaturation temperature for whey in solution ranges from 50 to 90°C for 30 min (McClements and Keogh, 1995; Ennis and Mulvihill, 2000). Thermal denaturation takes place at 50 and 75°C unfolding and unmasking the SH groups (Linden and Lorient, 1999). The level of denaturation and subsequent insolubility at pH 7 depends on heating temperature and time, and the pH of whey at heating (Ennis and Mulvihill, 2000). Whey in solution is heated up to 30 min and lost protein N as temperatures increased above 80°C, but we observed no significant change in protein N content after drying. Though the amount of protein denatured increased, with increasing

Table 2. Properties of WPI as function of extrusion temperature.

Extrusion cook temperature	Melt (°C)[1]	pH	Protein[2] (%)	Insoluble (%)	Digestibility (%)
35	39[d]	6.7[b]	90.7	28.4[c]	89.6[a]
50	48[c]	6.8[ab]	90.9	3.3[c]	88.2[ab]
75	66[b]	6.9[a]	91.7	77.7[b]	85.7[bc]
100	92[a]	7.0[a]	91.4	87.2[a]	84.5[c]

[a,b,c]Means with different letters within a column are significantly ($P < 0.05$) different.

[1]Extrusion melt temperature at the die.

[2]Protein percentage after drying. Properties of nonextruded WPI: pH 6.8, protein 88.9%, insoluble (denatured) 28.0%, and digestibility 87.7%.

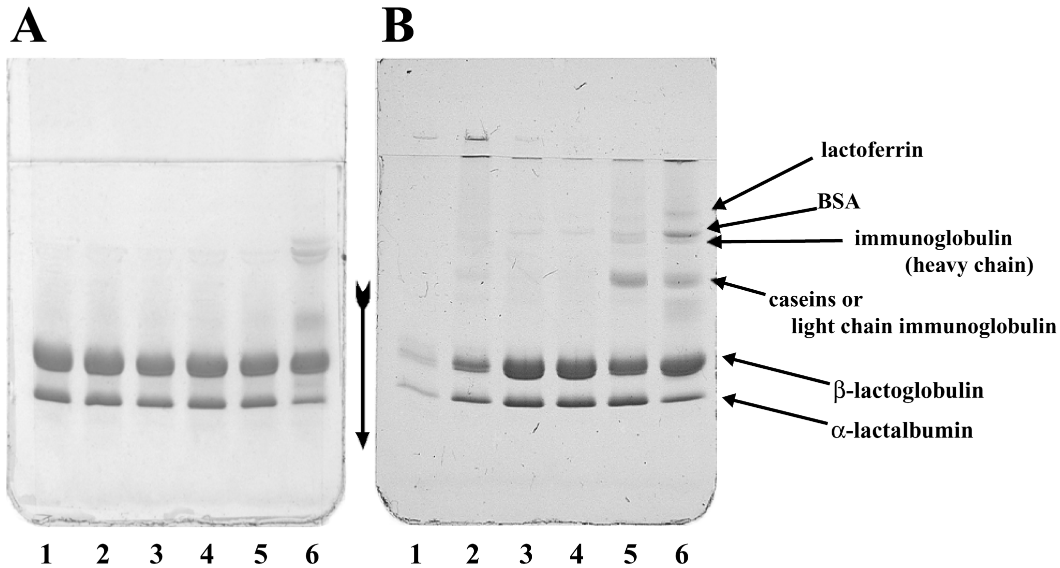

Figure 2. SDS-PAGE of extruded whey isolates. (A) With 2-mercaptoethanol; (B) without 2-mercaptoethanol. The lanes are: 1 = 100°C; 2 = 75°C; 3 = 50°C; 4 = 35°C; 5 = Native WPI; 6 = laboratory whey.

temperature, denaturation had minimal overall effect on protein digestibility. So, the interesting result is increased protein denaturation without a significant loss of digestibility due to extrusion below 100°C. Extrusion denaturation occurs in the short time order of 45 to 90 s within the extruder. The short time might explain why extrusion texturized WPI maintains its digestibility.

The WPI and variously heat-treated samples were compared by SDS-PAGE (Figure 2). The SDS gel of the variously denatured WPI, indicated minimal change in solubility (Figure 2). The SDS gels were initially developed without reducing reagent so the protein disulfide bonds are intact. The unreduced samples at 35 and 50°C show somewhat diminished bands for the higher molecular weight whey proteins (B). However, at 50 and 75°C samples were equivalent weight, and fainter than the native whey or whey proteins produced in the laboratory on the SDS gel (compare lanes 1 and 2 with 6 in Figure 2). In this respect, the SDS gels parallel the solubility data in that increased temperature decreases solubility in SDS alone, indicating sulfhydryl-disulfide crosslinking. When the samples were reduced thoroughly and all disulfide bonds cleaved, all the extruded whey samples at the different temperatures were similar to each other and to the initial WPI (A). Thus, extruding whey even at the highest temperatures does not affect the overall protein ratios. The native and extruded whey still have the same amount of the different proteins (Figure 2) and their total N values were similar (Table 2). Soy proteins and gluten are two systems that are generally extruded at high temperature and low moisture contents to form structured products. Their solubility is high, thus requiring that their hard-to-break disulfide bonds be dissolved with high solubility solvents such as β-mercaptoethanol and sodium dodecyl sulfate (SDS). Our denatured whey protein system behaved similarly to soy protein and gluten, showing a similar pattern of bonding and crosslinking. Whey isolates were most denatured by heat of different proteins extruded (Mohammed et al., 2000).

Physical functional properties of extruded WPI such as gel strength, foam volume, and stability were significantly affected at and above 75°C and were proportionally affected at lower preset temperatures. Greater than 30% moisture was needed to extrude the whey protein isolates, but the only significant change in mois-

Table 3. Physical properties whey protein isolate (WPI) as function of extrusion temperature.[1]

Extrusion cook temperature	Moisture (%)	Gel strength (N)	Foam volume (%)	Foam stability
35	42.5[a]	114.9[b]	298.1[ab]	29.8[ab]
50	40.9[b]	145.3[a]	301.9[a]	30.2[a]
75	42.6[a]	2.8[c]	173.3[b]	17.3[c]
100	38.9[c]	—[2]	77.1[c]	7.7[c]

[a,b,c]Means with different letters within a column are significantly ($P < 0.05$) different.

[1]Properties of nonextruded WPI: moisture 1.94%, gel strength 52.3 (N), foam volume 288%, and foam stability 28.7%.

[2]Value not reported.

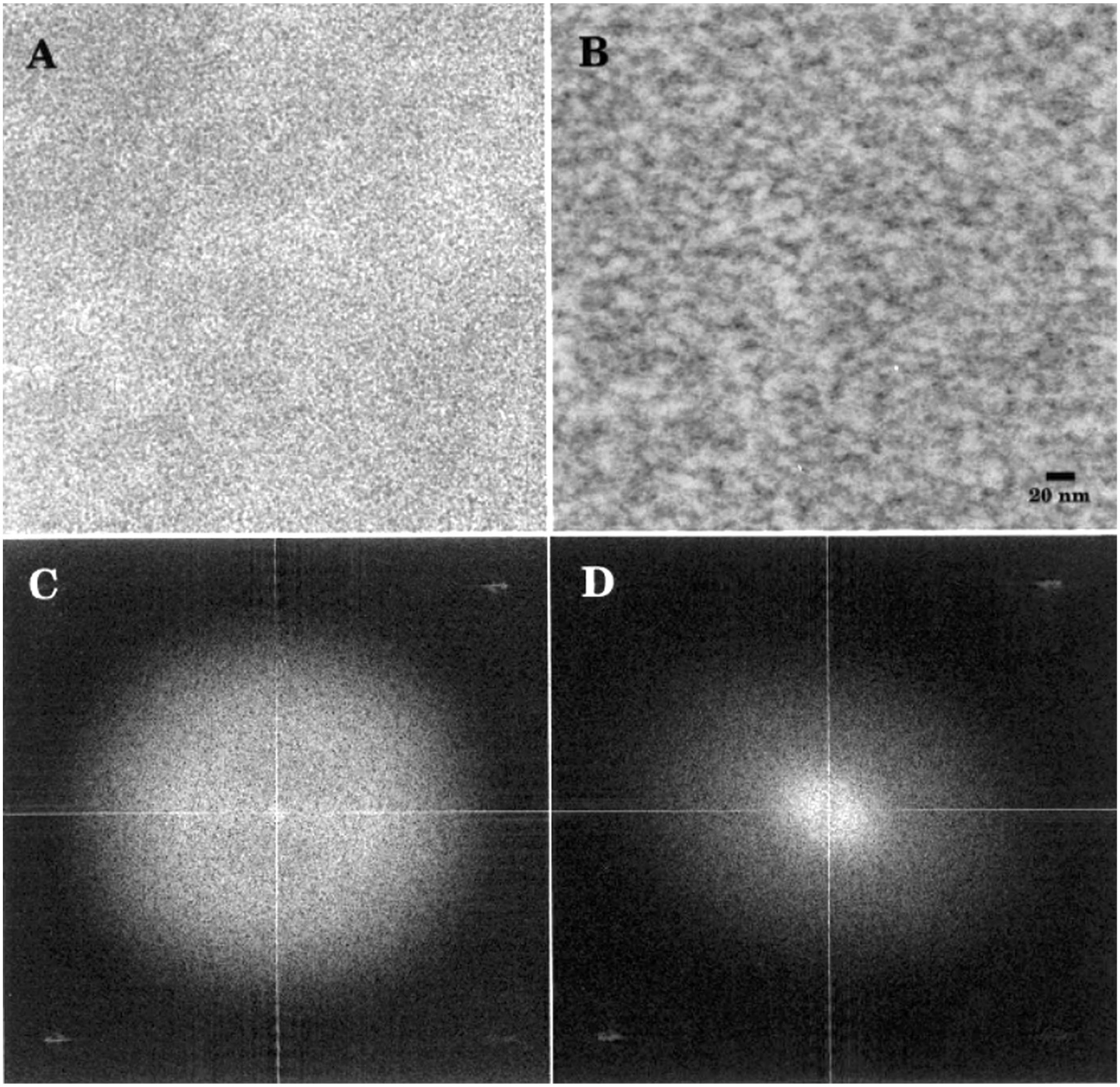

Figure 3. Transmission electron micrographs of whey protein isolates (WPI) positively stained with uranyl acetate and lead citrate: (A) Enlargement of denatured whey extruded at 100°C, as in Figure 1C; (B) enlargement of a selected protein-dense area of Figure 1B, undenatured paste of WPI; (C) fast Fourier transforms of electron density images, native and denatured (D).

ture of the extruded products occurred at 100°C (Table 3). Partial denaturation at temperatures between 35 and 50°C significantly increased gel strength, but at 75°C or higher complete loss of gelling property resulted. Foam volume remained high up to 50°C but decreased significantly ($P < 0.05$) after 75°C. Foam stability followed the same pattern as volume, being very stable for an hour below 50°C. This result is contrary to the report that WPI, heated to 80°C had little effect on stability (Phillips et al., 1990). However, with the addition of shear from the extruder, we observed significant loss of volume and stability.

Denatured whey protein isolate looks quite different from the nondenatured proteins at the ultrastructural

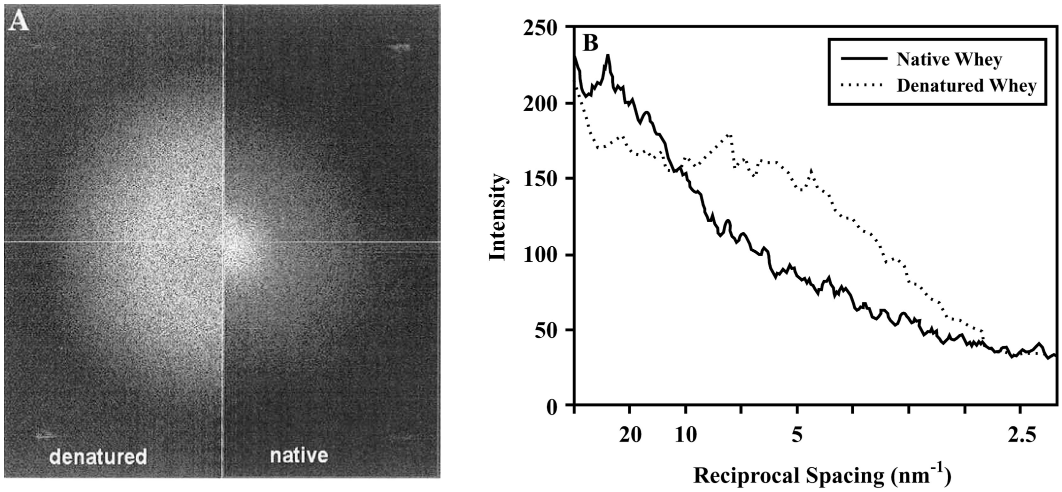

Figure 4. Electron-density mapping corresponding to the Fourier transforms (A) for denatured (extruded at 100°C) and native whey protein isolate (WPI), and (B) inverse reciprocal spacing of electron-density images of native and denatured WPI.

level (Figure 3). As sampled, denatured proteins (3A) (WPI extruded at 100°C) are densely packed with spacing of 2 to 6 nm, while nondenatured whey in the paste are loosely packed with a large spacing 200 to 350 nm (3B). The differences in fine structure of denatured and native whey protein are illustrated in Figures 3 and 4. In the “native” whey protein (40% slurry), the distribution of electron density surrounding the hydrating particles (Figure 1B) is an open network with clear, electron-lucent spaces ranging from 15 to 40 nm and irregular structures of electron density of similar dimensions. In contrast, the fine structure in segments where the whey proteins are completely denatured is limited to close-packed fine granules around 3 to 8 nm in diameter (Figure 3). The corresponding computed Fourier transforms indicate that images of extrudate containing native whey proteins consist mainly of low spatial frequencies, indicating structures with average spacings ranging from 15 to over 40 nm, whereas images of extrudate containing denatured whey proteins have little intensity at low spatial frequencies but high intensity corresponding to high spatial frequencies, relating to electron density changes ranging from about 3 nm to less than 10 nm (Figure 4). The constraint of extruding whey is keeping the temperature below the point where pyrosis will occur, as evidenced by relatively constant N content (Table 2). Texturized whey products are sometimes extruded at 150°C to form meat-like stringy structures (Lin et al., 2000). It is thus possible that whey proteins extruded at a temperature higher than 100°C would form very dense fibrous structures. We have seen evidence of fine structures with transmission electron microscopy images at 100°C in whey isolates. There is further evidence that texturized whey products could function as food adjuncts. In a consumer taste, extrusion texturized whey protein concentrate (80% protein) was shown to be comparable to texturized soy protein (Hale et al., 2002).

We have created structured networks in whey proteins using mild heat and shear to create reversible texturized whey proteins. By understanding the effects of shear on a molecular basis, ways of creating new functionality can be developed. This will enable development of extrusion parameters that permit controlled denaturation of whey proteins.

CONCLUSION

Extrusion processing denatured whey protein concentrates, WLAC and WPI, but the greatest amount of denaturing occurred with WPI. Denatured whey protein isolate retained its native protein value, functionality, and digestibility when extruded at 50°C or below; changes in functionality occurred at 75 and 100°C. Through careful selection of extrusion conditions, denatured whey proteins with unique functionality were produced. Texturization increased with temperature, but temperatures higher than 100°C may be needed to form texturized fibrous products from whey protein isolates. We show here that extrusion is an effective tool for denaturing whey proteins to create texturized products. Thermally denatured WPI is a unique ingredient that can be used in large amounts in nontraditional applications such as in acidified shelf-stable beverages.

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